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mutation confers an altered fatty acid composition in seeds of said plant.

Please add the following new claim:

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806  
D131  
66. (New) An isolated nucleic acid fragment comprising a sequence of at least about 20 nucleotides from a *Brassicaceae* or *Helianthus* delta-12 fatty acid desaturase gene having at least one mutation in a region of said desaturase gene encoding a Tyr-Leu-Asn-Asn-Pro amino acid motif, wherein said at least one mutation is effective for altering the fatty acid composition in *Brassicaceae* or *Helianthus* seeds and wherein said sequence includes said at least one mutation.

#### REMARKS

Claims 4, 11-26, 30 and 36 have been canceled. Claims 1, 5-7, 10, 27, 32, 35, 40 and 44-46 have been amended herein. New claim 66 has been added. Applicants request reconsideration and allowance of claims 1-3, 5-10, 27-29, 31-35, 37-46 and 55-66.

Claims 1 and 27 have been amended to recite that the mutation is in a region of the desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif. Support for these amendments can be found, for example, at page 13, line 31 through page 14, line 2.

Claims 5 and 10 have been amended to correct the dependency of the claims.

The term "wild-type amino acid" has been added to describe the sequence in claims 6, 32 and 40. Support for these amendments can be found, for example, at page 14, lines 16-22.

Claims 7 and 10 have been amended for grammatical clarity.

Claims 35, 44 and 46 have been amended to recite that the plants contain a full-length coding sequence of a desaturase gene. Support for these amendments can be found, for example, at page 12, lines 22-25.

Claim 45 has been amended to recite that the decreased  $\alpha$ -linolenic acid content is compared to  $\alpha$ -linolenic acid levels in corresponding seeds lacking the mutant genes. Support for this amendment can be found, for example, at page 12, lines 1-18.

New claim 66 recites a nucleic acid fragment of at least about 20 nucleotides in length from a *Brassicaceae* or *Helianthus* delta-12 fatty acid desaturase gene containing at least one mutation in a region of the desaturase gene encoding a Tyr-Leu-Asn-Asn-Pro amino acid motif. The mutation is effective for altering the fatty acid composition in *Brassicaceae* or *Helianthus* seeds. Support for this claim can be found, for example, at page 15, lines 12-17.

Applicants submit herewith an amendment to the Sequence Listing made in accordance with 37 C.F.R §1.825. These amendments, included in the substitute sheets and substitute copy of the computer readable form of the Sequence Listing, were described in the Preliminary Amendment of September 12, 1997. The source information (clone) for SEQ ID NO: 3 and SEQ ID NO:7 have been switched in the attached Sequence Listing. The T to A transversion at nucleotide 515 of SEQ ID NO:3 and the corresponding His to Leu amino acid change at amino acid 172, inadvertently introduced at nucleotide 563 and amino acid 188 in the previous amendment to the Sequence Listing, have been made in SEQ ID NO:3. No new matter is introduced by these amendments.

#### Objection under 35 U.S.C. §132

The Examiner has objected, under 35 U.S.C. §132, to the amendment filed on September 16, 1997, for introducing new matter into the specification. The Examiner stated that the amendment directed to changes in sequence data at page 14, lines 21 and 22 and page 25, lines 26 and 27 was not supported by the original disclosure. The amendment was seen as constituting new matter in the absence of a showing that the correct sequence was obtained from the same material via a clear chain of custody to an unambiguous source such as a deposit of the original material from which the invention was obtained.

As indicated in paragraphs 5 and 6 of the attached Declaration under 37 C.F.R. §1.132 of Guo-Hau Miao, the Fad2-F and Fad2-D sequences were incorrectly labeled during preparation of the Sequence Listing. This is evidenced by a comparison of the notebook pages containing these sequences with the sequences that were submitted to Ron Lundquist, Applicants' representative. See, paragraph 6 of Miao Declaration. Thus, the amendment submitted on September 16, 1997 did not contain any new matter. The Examiner is requested to withdraw the objection under 35 U.S.C. §132 and to enter the amendments at pages 14 and 25 with respect to the Sequence data.

Rejection under 35 U.S.C. §101

Claim 17 was rejected under 35 U.S.C. §101 as claiming the same invention as that of claim 6 of prior U.S. Patent 5,668,229. Applicants believe that the Examiner is referring to U.S. Patent 5,668,299 (the '299 patent). Applicants also believe that the Examiner is referring to claim 18 of the present application. Claim 18 has been canceled, thereby mooting this rejection.

The Examiner has rejected claims 11-17, 19-26 and 35-46 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 6, 16-18, 20 and 21 of the '299 patent. The Examiner asserted that the conflicting claims are not patentably distinct from each other because each is drawn to mutant *Brassica* or *Helianthus* plants that comprise fatty acid desaturase genes which confer altered levels of oleic and/or linoleic acid. The prior patent and the instant application were seen as comprising species of the genus of plants comprising altered levels of oleic and linoleic acid. Claims 11-26 have been canceled without prejudice. Applicants respectfully traverse with respect to claims 35-46.

Amended claims 35-45 are directed to *Brassicaceae* or *Helianthus* plants containing a delta-15 fatty acid desaturase gene with a mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif. Applicants submit that the '299 patent

contains no teaching or suggestion of delta-15 desaturases with mutations in the recited motifs and that it is very clearly hindsight to consider such a possibility.

Amended claim 46 is directed to a *Brassicaceae* or *Helianthus* plant containing a delta-12 desaturase gene with a mutation in a region encoding a Tyr-Leu-Asn-Asn-Pro amino acid motif. The '299 patent does not teach or suggest *Brassicaceae* or *Helianthus* plants containing a delta-12 desaturase gene having a mutation in a region encoding a Tyr-Leu-Asn-Asn-Pro amino acid motif.

It is only with hindsight in view of the present specification that claims 35-46 can be seen as obvious. Thus, the Examiner is requested to withdraw the obviousness-type double patenting rejection of these claims under 35 U.S.C. §101.

Rejection under 35 U.S.C. §112, first paragraph

The Examiner has rejected claims 1-46 and 55-65 under 35 U.S.C. §112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time the application was filed. The Examiner stated that the data presented in Tables 3-6 of the preliminary amendment indicate that at the time of filing, applicants did not have the claimed invention in their possession. Applicants respectfully traverse.

The specification clearly describes desaturase genes with mutations in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif. For example, the specification at page 14, line 2 through page 15, line 17, and in Tables 1-6, discusses the sequence of conserved His-Xaa-Xaa-Xaa-His motifs in desaturases from various species and provides examples of mutated motifs. The sequence changes presented in the Preliminary Amendment do not diminish the significance of the discovery that conserved His-Xaa-Xaa-Xaa-His motifs are an important target in rendering these desaturases non-functional. This discovery was clearly in the Applicants' possession as of the filing date in view of the

description in the specification. The Examiner is requested to withdraw this rejection under 35 U.S.C. §112, first paragraph.

The Examiner has rejected claims 1-3, 5, 8-9, 12-15, 19, 27-29 and 35 under 35 U.S.C. §112, first paragraph. The Examiner stated that while the specification is enabling for mutations in the amino acid motif His-Xaa-Xaa-Xaa-His which result in non-functional delta-12 and delta-15 fatty acid desaturase genes from *Brassica* or *Helianthus*, it does not reasonably provide enablement for mutations in other regions of the genes.

Applicants have amended independent claims 1 and 27 to recite that the mutation occurs in a region of the desaturase gene that encodes a His-Xaa-Xaa-Xaa-His amino acid motif.

The Examiner asserted that there was no linkage of the mutations exemplified in the specification with the limitation to "at least 20 nucleotides" having "... at least one mutation" as found in claim 1, for example. Claim 35 has been amended to recite that the plant contains a full-length coding sequence of the desaturase gene. Claims 12-15 and 19 have been cancelled. Applicants respectfully traverse with respect to claims 1-3, 5, 8-9, and 27-29.

The claimed nucleic acid fragments are from a region of a desaturase encoding a His-Xaa-Xaa-Xaa-His amino acid motif. These regions are conserved among the sequences of various desaturase genes. See, specification, Tables 1-6.

As described in the specification, at page 27, lines 22-32, nucleic acid fragments of at least 20 nucleotides in length can be used, for example, as markers in plant genetic mapping and plant breeding programs. Marker-assisted breeding techniques may be used to identify and follow a desired fatty acid composition during the breeding process. An example of marker-assisted breeding is the use of PCR primers that specifically amplify a sequence containing a desired mutation in delta-12 or delta-15 desaturase. For example, mutations within the His-Xaa-Xaa-Xaa-His amino acid motif of delta-12 or delta-15 desaturase could be specifically amplified. Thus, the limitation

of "at least 20 nucleotides in length" of amended claims 1-3, 5, 8-9, 12-15, 19 and 27-29 is enabled under 35 U.S.C. §112, first paragraph.

The Examiner has rejected claim 17 under 35 U.S.C. §112, first paragraph, as the plant designated as ATCC accession number 40811 must be readily available to practice the claimed invention. Applicants believe that the Examiner is referring to claim 18. Applicants have canceled claim 18.

The Examiner is requested to withdraw the rejections under 35 U.S.C. §112, first paragraph.

Rejection under 35 U.S.C. §112, second paragraph

The Examiner has rejected claims 1-46 and 55-65 under 35 U.S.C. §112, second paragraph, for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner asserted that the limitation of 20 nucleotides appeared to be an arbitrary size with no direct linkage to the function of the gene or mutation in the gene. The Examiner asserted that to a person having skill in the art, a mutation that affects an amino acid sequence need not affect the amino acid function, and that the specification clearly teaches the limitation of mutations that make the enzyme non-functional. The Examiner asserted that all mutations must be defined in terms of what is considered to be "wild-type" of a gene, and that the specification teaches modifications in the motif HXXXH, which do not encompass mutations resulting from up or down regulation.

Applicants have canceled claims 4, 11-26, 30 and 36. Applicants respectfully traverse this rejection with respect to the remaining claims. Claims 35 and 46 have been amended to recite that the plants contain a full-length coding sequence of a desaturase gene having at least one mutation in a region encoding either a His-Xaa-Xaa-Xaa-His or Tyr-Leu-Asn-Asn-Pro amino acid motif.

As described in the specification, at page 12, lines 1-18, the described mutations render the gene product non-

functional, as inferred from a decreased level of reaction product and an increased level of substrate in plant tissues expressing the mutant sequence in comparison with the corresponding levels in plants expressing the wild-type sequence. Thus, a non-functional gene product results in an altered fatty acid composition as recited in the claims.

Amended claims 1-3, 5, 8-9 and 27-29 relate to isolated nucleic acid fragments from desaturase genes that are at least 20 nucleotides in length and encode a region having a mutation in a His-Xaa-Xaa-Xaa-His amino acid motif. As discussed above, nucleic acid fragments of this length can be used as probes or primers for identifying desaturase genes that contain mutations within the His-Xaa-Xaa-Xaa-His amino acid motif. Thus, the length of these fragments is not an arbitrary size and need not be linked to the function of the gene.

The Examiner has rejected claims 7, 17, 33 and 41 under 35 U.S.C. §112, second paragraph, for being improperly dependent in that claim 6 was limited to a modified motif of amino acids that comprise a specific amino acid of the plant of claim 5, while the plant of claim 7 comprises a different motif and yet is dependent from the plant of claim 6. Claims 17, 33 and 41 were seen as presenting the same problem of dependency. Applicants respectfully traverse.

Claims 6, 32 and 40 recite the wild-type amino acid His-Asp/Glu-Cys-Gly-His motif. Claims 7, 33 and 41 further restrict the claims from which they depend by reciting a particular mutated amino acid sequence, and are thus proper dependent claims. Claim 17 has been canceled, thereby mooted this rejection with respect to claim 17.

The Examiner has rejected claim 45 under 35 U.S.C. §112, second paragraph, as there is no reference to what the decreased level of fatty acid is measured to. Claim 45 has been amended to recite that the decreased linolenic acid content is in comparison with  $\alpha$ -linolenic acid levels in seeds lacking the mutant genes.

The Examiner is requested to withdraw the claim rejections under 35 U.S.C. §112, second paragraph.

Rejection under 35 U.S.C. §102

The Examiner has rejected claims 1-5, 8-9, 11-12, 14-15 and 19-21 under 35 U.S.C. §102(b) as being anticipated by Edwards et al. (WO 94/11516, abstract and sequence analysis). Edwards was seen as disclosing isolated nucleic acid fragments, microsomal gene products and whole genes that comprise at least one mutation in the delta-12 fatty acid gene. The Examiner stated that the fatty acid desaturase clone seq. registry no 158283-28-4 discloses mutations in this motif as HECGH, HRRHHH and HVAHH, while registry no. 158283-32-0 discloses HDCGH and HRRHH, and as such anticipate the claimed invention. Applicants respectfully traverse this rejection. Applicants believe that the Examiner is referring to Lightner et al., WO 94/11516. Applicants also believe that the Examiner is referring to an HRRHH motif in seq. registry no. 158283-28-4.

Lightner et al. describe examples of wild-type delta-12 desaturase genes from *Arabidopsis*, *Brassica*, soy and maize, but do not describe a mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, let alone a mutation that affects fatty acid composition.

In contrast, pending claims 1-3, 5 and 8-9 relate to mutated delta-12 desaturase genes. The present specification describes motifs that are suitable for mutation, including the wild-type HECGH, HVAHH, HDCGH and HRRHH motifs in delta-12 desaturases. Specification, pages 15-16, Tables 1-3. Preferred mutations occur in regions containing a conserved His-Xaa-Xaa-Xaa-His motif. Specification, page 13, line 31 through page 14, line 22. These motifs are found in different regions of the *Brassica*, *Arabidopsis*, soybean, corn and *Ricinus* desaturases, as summarized below in Table I.

Table I  
Wild-type Delta-12 desaturase Motifs

Motif	Species	Amino acid position
HECGH	<i>Brassica</i>	105
	<i>Arabidopsis</i>	105
	Soybean	101
	Corn	111
HRRHH	<i>Brassica</i>	141
	<i>Arabidopsis</i>	141
	Soybean	137
	Corn	147
	<i>Ricinus</i>	41
HDCGH	<i>Ricinus</i>	5
HVAHH	<i>Brassica</i>	316
	<i>Arabidopsis</i>	315
	Soybean	311
	Corn	322

Since Lightner et al. does not disclose mutated desaturase sequences, the reference does not anticipate the claimed invention. The Examiner is requested to withdraw the rejection of claims 1-3, 5 and 8-9 under 35 U.S.C. §102(b) over Lightner et al.

The Examiner has rejected claims 27-31, 34-36, 38-39 and 42-43 under 35 U.S.C. §102(b) as being anticipated by Browse et al. (WO 93/11245). Browse et al were seen as disclosing the presence of the motif HDCDH, HRTTH, HHHGH, and HVIHH in seq. registry no. 149955-98-6, and HDCGH, HRTTH, HHHGH and HVIHH in Seq. registry no. 149955-98-6, both isolated from *Brassica napus* delta-15 desaturase which comprise mutations from the claimed motif and as such anticipate the claimed invention. Applicants respectfully traverse this rejection. Applicants believe that the Examiner is referring to HDCGH in Seq. registry no. 149955-98-6.

Browse et al. describe examples of a wild-type *Arabidopsis* delta-15 fatty acid desaturase, as well as the corresponding rape, soybean and corn wild-type desaturases. Browse et al. do not teach a mutation in a His-Xaa-Xaa-Xaa-His

amino acid motif. In contrast, the presently claimed invention relates to mutated delta-15 desaturase genes.

The present specification describes motifs that are suitable for mutation, including the wild-type HDCGH and HRTTH motifs in delta-15 desaturases. Specification, pages 17-18, Tables 5-6. Preferred mutations occur in regions containing a conserved His-Xaa-Xaa-Xaa-His motif. Specification, page 13, line 31, through page 14, line 2 and page 13, lines 22-29. The HHHGH and HVIHH motifs described by Browse et al. are additional examples of wild-type His-Xaa-Xaa-Xaa-His motifs. These motifs are found in different regions of the *Brassica*, *Arabidopsis*, soybean, corn and *Ricinus* desaturases, as summarized below in Table II.

Table II  
Wild-type Delta-15 desaturase Motifs

Motif	Species	Amino acid position
HDCGH	<i>Brassica</i>	95
	<i>Arabidopsis</i>	101
	Soybean	100
HRTTH	<i>Brassica</i>	131
	<i>Arabidopsis</i>	137
	Soybean	136
HHHGH	<i>Brassica</i>	255
	<i>Arabidopsis</i>	261
	Soybean	260
	Corn	121
HVIHH	<i>Brassica</i>	298
	<i>Arabidopsis</i>	304
	Soybean	303
	Corn	121

Since Browse et al. do not disclose mutated desaturase sequences, they do not anticipate the claimed invention. The Examiner is requested to withdraw the rejection of claims 27-29, 31, 34-36, 38-39 and 42-43 under 35 U.S.C. §102(b) over Browse et al., WO 93/11245.

Rejection under 35 U.S.C. §103

The Examiner has rejected claims 1-5, 8-15, 18-31, 34-39, 42-45 and 55-65 under 35 U.S.C. §103 as being unpatentable over Lightner et al. (WO 94/11516) and Browse et al. (WO 93/11245) in view of Pleines et al. The Examiner alleged that neither Lightner or Browse disclose the intended detection of the claimed motif as the means of action of fatty acid modification, or the method of mutant selection as that disclosed in the application. However, the modification of fatty acid content via mutagenesis and selection was seen as being taught by Pleines et al. Pleines was seen as disclosing modifications in the  $C_{18:1}/C_{18:3}$  oil seed ratio via mutagenesis and interspecific crosses to lead to improved food value for rapeseed oil. Applicants respectfully traverse.

Lightner et al. and Browse et al. disclose sequences of wild-type delta-12 and delta-15 desaturase genes from various species. Lightner et al. and Browse et al. do not teach or suggest that mutations in a His-Xaa-Xaa-Xaa-His amino acid motif would result in altered fatty acid composition.

Pleines et al. describe attempts to select plant lines with reduced  $C_{18:3}$  levels by breeding, and suggest that rapeseed oil for food consumption could be improved by mutagenic treatments and interspecific crosses. Mutations in a His-Xaa-Xaa-Xaa-His motif of a delta-12 or delta-15 desaturase are not disclosed. The pending claims relate to at least one mutation in a region of a fatty acid desaturase gene encoding a His-Xaa-Xaa-Xaa-His amino acid motif. Although mutagenesis may be suggested by Pleines et al., the combined art does not suggest which regions of these genes could be mutated to achieve alterations in fatty acid compositions. Therefore, Pleines et al. do not remedy the deficiencies of Lightner et al. and Browse et al.

It is only with hindsight in view of the present specification that the claimed invention would be seen as obvious. In view of the above, it is submitted that claims 1-3, 5, 8-10, 27-31, 34-39, 42-45 and 55-65 are patentably nonobvious.

The Examiner is requested to withdraw the rejections of the claims under 35 U.S.C. §103.

In light of the claim amendments and remarks presented above, it is submitted that all pending claims are in condition for allowance and such action is respectfully requested.

The Examiner is invited to telephone the undersigned agent if it is felt that such would advance prosecution.

Please charge any additional fees, or make any credits, to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 8/1/82

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